ing or is it only engaged in protein-protein interactions? Is the increase in Ars2 levels observed during the transition from cell quiescence to proliferation reflected in expression of miRNAs dependent on Ars2 for correct processing? And if so, what sequence or structure motif causes these miRNAs to be responsive to Ars2? Finally, given the small number of genes screened for involvement in viral resistance, how many other regulatory components are still waiting to be identified? With a growing understanding of the mechanisms behind small RNA pathways the new challenge in RNA biology is to understand how the silencing machinery is interconnected with general RNA metabolism. We have certainly not seen the last canonical RNA metabolic factor crossing over into the miRNA field.

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A New Therapeutic Target for Leukemia Comes to the Surface

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DOI 10.1016/j.cell.2009.07.005

Expression of the cell-surface protein CD47 allows some normal cells to avoid phagocytosis by macrophages. In this issue, Jaiswal et al. (2009) and Majeti et al. (2009) show that elevated CD47 expression by leukemic stem cells inhibits macrophage activity and is an indicator of poor prognosis for patients with acute myeloid leukemia.

The cancer stem cell hypothesis postulates that the progeny of a small pluripotent population of malignant stem cells makes up the bulk of a malignant tumor. Among cancers, acute myeloid leukemia (AML) has the most clearly defined population of stem cells—known as leukemic stem cells (LSCs)—that is responsible for AML persistence and relapse. Therefore, there is considerable interest in identifying therapies that specifically target this particular population of cells. Leukemic stem cells and their normal counterpart, the hematopoietic stem cells (HSCs), show remarkable resilience to the direct cytotoxic and indirect inflammatory effects of chemotherapy. This results, in part, from their ability to circulate freely in the peripheral blood and repopulate the bone marrow stem cell niche while avoiding phagocytosis by activated macrophages (Figure 1). In this issue of *Cell*, Jaiswal et al. (2009) and Majeti et al. (2009) show that the immunoglobulin-like protein CD47 expressed by LSCs acts as an inhibitor of macrophage activity and directly promotes enhanced survival and engraftment of LSCs (Figure 1). Furthermore, they demonstrate that high levels of CD47 on LSCs isolated from patients with AML are predictive of leukemia resistance to therapy, possibly reflecting the ability of LSCs to avoid macrophage-mediated control.

CD47 is a membrane protein that functions in neutrophil trafficking, T cell costimulation, and neuronal regeneration (Matozaki et al., 2009). CD47 also interacts with a specific macrophage receptor, SIRPα, to downregulate the phagocytic potential of macrophages (van den Berg and van der Schoot, 2008). Given that mobilized HSCs in the blood stream come into contact with macrophages of the reticulo-endothelial

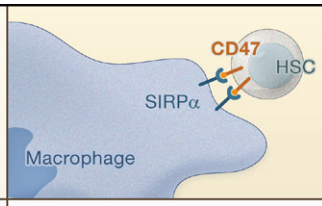
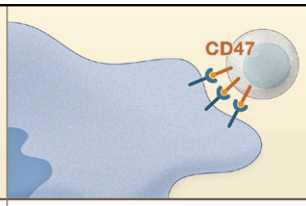
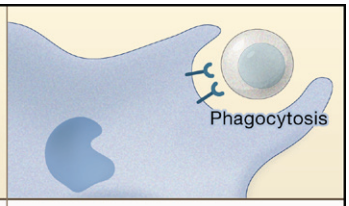
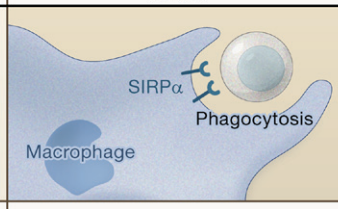
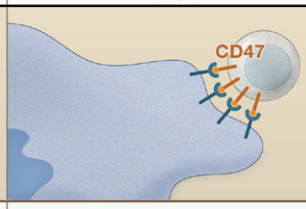
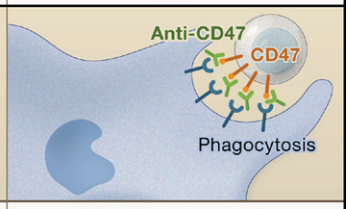
Normal hematopoietic stem cell			
Setting	Homeostatic maintenance	Inflammation/mobilization	HSC senescence
CD47 expression	Physiological	Increased	Reduced/absent
Macrophage activity	Physiological	Increased	Physiological
Net result	No phagocytosis	No phagocytosis	Increased phagocytosis
Experimental transplantability	Normal	High	Low
Leukemic stem cell			
Setting	AML with good prognosis	AML with poor prognosis	AML with poor prognosis treated with anti-CD47
CD47 expression	Low	High	High (but blocked)
Macrophage activity	Physiological	Suppressed	Physiological or increased
Net result	Enhanced phagocytosis	No phagocytosis	Enhanced phagocytosis
Experimental transplantability	Low	Very high	Low
Clinical prognosis	Good	Very poor	Improved

Figure 1. CD47 in Hematopoietic Stem Cells and Leukemic Stem Cells

The expression of CD47 by normal hematopoietic stem cells (HSC) varies depending on the physiological context. The CD47 receptor SIRPα is expressed by macrophages and interacts with HSC CD47 in settings of homeostatic maintenance, inflammation/sepsis, and possibly HSC senescence with differing outcomes. Similarly, leukemic stem cells (LSC) in acute myeloid leukemia (AML) interact with macrophages and express different levels of CD47 in different clinical settings. The level of CD47 expression correlates with the prognosis of AML patients, with lower CD47 expression found in those with a good prognosis (for example, harboring the cytogenetic marker t(8;21)) and higher CD47 expression found in those with a poor prognosis (for example, marked by FLT3-ITD expression). The treatment of patients with antibodies against CD47 that block its interaction with SIRPα may alter the clinical outcome by promoting phagocytosis and clearance of LSCs.

system (in the spleen and bone marrow) but escape phagocytosis, it is possible that survival of normal HSCs is at least partially dependent on their upregulation of CD47 expression (Figure 1).

Jaiswal and colleagues explore the expression of CD47 on normal HSCs mobilized after chemotherapy by granulocyte-colony stimulating factor (G-CSF) or the endotoxin lipopolysaccharide (LPS). They hypothesize that upregulation of CD47 expression is necessary for HSC survival in the G-CSF or LPS-induced inflammatory environment where there is increased macrophage phagocytic activity. Indeed, they find that HSCs expressing only one copy of the *CD47* gene are more poorly transplanted in comparison to HSCs expressing both copies of the *CD47* gene. This decreased transplantation fitness is due to increased clearance of the stem cells by macrophages. As normal HSCs have much in common with myeloid LSCs, Jaiswal et al. also examine the expression of CD47 on isolated populations of LSCs. Beyond confirming an earlier report of increased expression of CD47 in leukemic bone marrow (Traver et al., 1998), the authors find that cells from an isolated putative myeloid LSC population have increased expression of CD47 relative to normal HSCs. This suggests that LSCs are likely to enjoy enhanced survival due to decreased clearance by macrophages (Figure 1). LSCs isolated from a range of myeloid malignancies in patients also display elevated CD47 expression. Strikingly, those patients with the most malignant myeloproliferative disorders (chronic myeloid leukemia in blast crisis and AML) uniformly demonstrate the highest expression of CD47. Other myeloid proliferative disorders that do not have an expanded malignant stem cell population (including polycythemia, myelofibrosis, and essential thrombothycemia) did not show increased CD47 expression in their stem cell populations. These clinical data and other experimental data presented in the studies suggest that CD47 is not simply a differentiation marker. Instead, they strongly implicate increased CD47 expression as an attribute of the highly malignant LSC populations found in chronic myeloid leukemia in blast crisis and AML, suggesting that elevated levels of CD47 expression could underlie the rapid clinical course and incurability of the majority of these patients.

In light of the findings of Jaiswal et al., an obvious hypothesis is that in clinical presentations of AML, high expression of CD47 should result in the increased "escape" of LSC populations from macrophage-mediated surveillance. Indeed, Majeti et al. make this observation when examining LSCs from AML patients

where the disease was refractory to or relapsed shortly after remission induction chemotherapy. Currently, AML prognosis is best determined by cytogenetic analysis of leukemic blasts (Byrd et al., 2002). In cases where this is normal, the next step is to look for the presence of molecular abnormalities such as the expression of fms-related tyrosine kinase 3-internal tandem repeats (FLT3-ITD) (which confers a poor prognosis) or nucleophosmin-1 (which confers a better prognosis in the absence of FLT3-ITD expression) (Schlenk et al., 2008). Using well-documented criteria for stem cell identification, Majeti et al. report that the predictive value of CD47 expression in the LSC population is independent of most known prognostic indicators. An important exception to this is the AML subgroup harboring the cytogenetic abnormality t(8;21) (a chromosomal translocation between chromosome 8 and 21), which is associated with a better prognosis (Figure 1). The AML subgroup expressing the poor prognosis molecular marker FLT3-ITD is also an exception and shows the highest levels of CD47 expression (Figure 1).

Majeti et al. find that AML patients can be stratified into distinct groups based on CD47 expression. Survival (both disease-free and overall) is markedly longer in patients expressing low levels of CD47. This association also remains significant in a multivariate analysis of age, FLT3-ITD status, and CD47 expression, providing a clinical context to the in vitro and animal model studies of Jaiswal et al. Majeti and colleagues further show that consistent with findings in mice, CD47 expression on human LSCs results in decreased phagocytosis by macrophages, an effect that can be reversed by the presence of antibodies directed against CD47 (Figure 1) or SIRPα (the CD47 receptor on macrophages). Notably, normal HSCs treated with antibodies against CD47 did not seem susceptible to macrophage-mediated phagocytosis, indicating that inhibition of the CD47-SIRPα interaction is insufficient to induce phagocytosis of healthy HSCs. This suggests that another signal for engulfment exists on LSCs, "pre-priming" them for phagocytosis when CD47 cannot interact with SIRPα. Given that the in vivo viability of healthy HSCs is not impaired when CD47 is blocked by a monoclonal antibody, it may be possible to promote the clearance of LSCs without any detriment to bone marrow repopulation by normal HSCs. Thus, the authors provide the first glimpses of how a prosurvival signal for LSCs may be clinically subverted as a potential therapeutic target for leukemia (Figure 1).

Some questions remain unanswered by the studies of Jasiwal et al. and Majeti et al. For example, what is the normal physiological function of the interaction between normal HSCs and bone marrow or splenic macrophages? Could the interactions between bone marrow macrophages and HSCs expressing physiological levels of SIRPα and CD47, respectively, contribute to the HSC niche? Perhaps the expression of CD47 is a surrogate marker of HSC physical fitness that wanes as the HSC ages so that cells beyond an "expiration date" can be disposed of by tissue macrophages in a manner analogous to the clearance of damaged and senescent erythrocytes in the spleen. Bone marrow macrophages may, therefore, perform a normal homeostatic function by providing macrophage-derived survival signals to HSCs via the interaction between CD47 and SIRPα and may only switch to phagocytosis when CD47 expression drops below a certain level. The high expression of CD47 by malignant LSCs may represent another example by which prosurvival signals are hijacked by a malignant process to avoid normal immunosurveillance. Although little is known regarding host immunosurveillance of primitive pluripotent tumor stem cell populations, it has been proposed that the immune system may selectively "edit" the malignant cell population by destroying more susceptible cells, thereby producing a pool of malignant cells that are resistant to immunological recognition and clearance (Swann and Smyth, 2007). Patients who harbor LSCs with high expression of CD47 may have already undergone this process in which their low (and potentially less malignant) LSCs have been cleared by endogenous macrophages, leaving the more resistant LSCs that are ultimately responsible for poor clinical outcomes. A syngeneic mouse model of AML, where the role of CD47 and the safety of a neutralizing antibody can be fully explored, should dovetail with further clinical observations and trials to extend the major advances made by these two studies. These discoveries provide tangible avenues for the application of new therapies that will enhance the clearance of LSCs in vivo and ultimately lead to improved patient outcomes in leukemia.

ACKNOWLEDGMENTS

M.J.S. is supported by a National Health and Medical Research Council of Australia Research Program Fellowship.

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